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SASKATCHEWAN WHEAT BREEDING PROGRAMS ADDRESS THE FUSARIUM HEAD BLIGHT THREAT

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FINAL REPORT

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ABSTRACT

Funding from this project contributed to efforts to improve Fusarium Head Blight resistance in the AAFC, Swift Current and the Crop Development Centre, University of Saskatchewan spring wheat breeding programs and resulted in longer-term funding being received from another agency. Selection based on results of field disease tests at Carman and Minto, Manitoba during 2000 to 2003 caused a progressive increase in resistance in the breeding material of each program. To further assist the breeding program, studies to identify new sources of resistance and greenhouse screening methodology, to rapidly develop new breeding populations using these sources through use of doubled haploid technology, and to identify molecular markers for the Sumai 3-based resistance to permit rapid identification of resistant lines. Each of these studies achieved their goals.

EXECUTIVE SUMMARY

INTRODUCTION

This project involved eight spring wheat breeding programs, four located at the Crop Development Centre, University of Saskatchewan, Saskatoon and four at the Semiarid Prairie Agricultural Research Centre, AAFC, Swift Current. These programs cover the CWRS, CPS, CWES and CWAD classes of spring wheat. The project was administered by Dr. G.R. Hughes at the University of Saskatchewan, but each of the co investigators and the AAFC - SPARC collaborating scientists operated independently.

PROJECT OBJECTIVES

(a) To establish a field disease nursery to evaluate the resistance of elite germplasm and wheat breeding material to Fusarium Head Blight (FHB);
(b) To determine the FHB reaction of resistance sources and selected advanced breeding lines under controlled conditions;
(c) To facilitate use of these parental sources in developing FHB resistant breeding populations using the doubled haploid or other accelerated breeding technology; and
(d) To use molecular marker-assisted selection to speed up the incorporation of genetic resistance to FHB.

1. FIELD SCREENING OF GERMPLASM AND BREEDING MATERIAL FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

Materials and Methods

Field disease nurseries at two sites in Manitoba,: (1) at Carman, in collaboration with Dr. Anita Brule-Babel, University of Manitoba, and (2) at Minto, conducted by AgQuest Inc., were used in some or all years of this project to screen lines from the spring wheat breeding programs of the Crop Development Centre, University of Saskatchewan and of the Semiarid Prairie Agricultural Research Centre (SPARC), AAFC, Swift Current. The breeding material screened was made up mostly of advanced and doubled haploid lines from crosses made to incorporate FHB resistance into the breeding germplasm. Much of this material had been subjected to prior selection for various agronomic traits and rust resistance. The material was planted in unreplicated hill or 1-m row plots with resistant and susceptible checks included at regular intervals throughout the nursery.

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In addition to relying on natural infection by FHB, both nursery sites were artificially inoculated with infected maize kernels and /or spraying with a spore suspension of *Fusarium graminearum*, usually three times at Carman and five times
at Minto throughout July. Mist irrigation was applied regularly to enhance disease development. Disease rating was done on a visual basis about three weeks after the last inoculation and a second rating was done at Minto about one week later. In 2000 at Carman, plots were rated for average % head infection, but in subsequent years each plot was rated for average % head infection and % heads infected and these two values were multiplied to give the FHB Index for that plot. At Minto, plots were rated on a 1-5 scale, where 1 = no recognizable infection; 2 = some florets infected on some plants; 3 = more than half the heads showing 25-50% infection; 4

= most heads showing 50% or more infection; 5 = all heads on a plant completely infected or dead.

RESULTS

1. Year 2000 Nurseries
(a) Carman Nursery

CDC Breeding Lines

Eight hundred doubled haploid (DH) lines from seven crosses, hills of 800 plants selected from 20 bulk populations tested in 1999 and eight new bulk populations were grown. Of 165 Doubled Haploid (DH) lines tested in both the 1999 and 2000 Manitoba field nurseries, 14% (22 lines) rated 5% (highly resistant) in both years (Table 1). These lines were yield tested in 2001. Of the other DH lines that were tested in 2000, 266 rated 15% or less FHB head infection and have been included in the 2001 nursery for a second year of testing to confirm their resistance.

Table 1. Summary of FHB ratings for Doubled Haploid lines tested at Carman in 2000

% Head Infection	Number of DH Lines in each FHB Rating Category, Carmen, 2000
0 to 5	132
6 to 10	180
11 to 20	207
21 to 30	135
31 to 40	71
> 40	55
Total	**780**
Check Cultivar	**Mean % Head Infection of Check Cultivars**
ND2710 (R)	2 ∀ 0.7
BW205	15 ∀ 0.0
AC Elsa	18 ∀ 2.5
CDC Teal (S)	33 ∀ 6.7

Approximately 65 single-plant hills showed less than 5% FHB infection and are undergoing agronomic testing in 2001. About 600 selections were made from heads harvested randomly from the eight bulk populations. These selections were based on visual evaluation of FHB-damaged kernels (shrunken and "tombstone" kernels) from a head; heads with no or a low frequency (5%) of FHB-damaged kernels were selected.

SPARC Breeding Lines
Entries in the 2000 FHB Evaluation Nursery included 5,300 hills and 690 bulk populations from the CWRS program, 1,400 hills from the CPS program, 210 rows from the CWES program and both rows and hills from the CWAD program. Selections with FHB resistance at least equal to AC Barrie were made and FHB testing of these selections will be repeated in 2001.

(b) 2000 Minto Nursery

CDC Breeding Lines
A total of 188 DH lines from two CWRS cross populations were included in the Minto nursery. Fifty-eight lines were retained for further testing.

Nine F2 CPS populations were grown as bulks and 200 heads were selected from each population and visually evaluated for the amount of FHB-damaged kernels. Lines with plump seeds were selected from six of the nine populations and are being tested again in 2001.

A collection of 900 tetraploid and diploid wheat accessions were also grown. The accessions included *Triticum carthlicum*, *T. dicoccoides*, *T. dicoccum*, *T. polonicum*, *T. timopheevii*, *T. turanicum*, *T. turgidum* and *T. monococcum*. Highly resistant accessions were found in all species although their frequency was lowest in *T. polonicum* and T. *turanicum* (Table 2).

2. **Year 2001 Nurseries**
In late 2000, this project received matching funding from WGRF to run a Joint/Collaborative FHB nursery at the University of Manitoba's Carman site. The institutions involved are the Crop Development Centre, Saskatoon, University of Manitoba, University of Alberta, AAFC - Swift Current and AAFC - Winnipeg. As a result of this increased funding level, our capabilities for field testing for FHB resistance have been increased considerably. It is important to recognize that the funding received from the Saskatchewan Agriculture Development Fund for this current project was a key factor in obtaining this additional support. In turn, these two sources of funding were influential in obtaining funding from ARDI, Manitoba in early 2001 to conduct epidemiological and pathogen variability studies involving University of Manitoba, University of Saskatchewan and AAFC personnel. Thus, the original grant from SADF has played a critical role in providing opportunities for a truly integrated research effort on FHB in western Canada.

(a) 2001 Carman Nursery

CDC Breeding Lines
Over 1,200 CWRS doubled haploid (DH) lines were tested (Table 3). Approximately 700 of these were evaluated concurrently for agronomic traits in unreplicated trials at Saskatoon and after selection for FHB resistance, agronomic performance and quality evaluation, 41 were retained. These 41 lines were increased in our New Zealand winter nursery and are being grown in multi-site agronomic tests in 2002. Included in the other 500 DH lines tested were 290 lines being tested for a third year. Of these, 16% (47) showed an FHB rating of # 5% with 20 lines receiving a rating of # 2%, indicating a high level of resistance. For comparison, the resistant check ND 2710 rated 5%,

AC Barrie, the intermediate resistant check, rated 15% and CDC Teal, the susceptible check, rated 25%. All lines were tested for rust resistance at Saskatoon in 2001.
Seed samples of F3 families from of nine CPS populations were examined for low incidence of FHB-damaged kernels. Seven hundred and fifty lines were selected and were also increased in the off-season winter nursery. These selections will be evaluated as F5 hills at Saskatoon in 2002.

Table 2. FHB Reaction of Accessions of Tetraploid and Diploid Wild Wheat Species in the 2000 Minto Nursery

Species	Rating date	Number of plants in each FHB reaction class[a]				
		1	2	3	4	5
T.carthlicum	August 12, 2000	14	44	11	1	1
	August 28, 2000	0	0	10	15	46
T. dicoccoides	August 12, 2000	14	10	5	2	0
	August 28, 2000	1	3	13	5	12
T. dicoccum	August 12, 2000	91	83	32	11	0
	August 28, 2000	11	49	63	54	54
T. monococcum	August 12, 2000	87	5	2	0	0
	August 28, 2000	10	44	31	15	3
T. persicum	August 12, 2000	0	0	1	0	0
	August 28, 2000	0	0	0	0	1
T. polonicum	August 12, 2000	10	20	18	1	11
	August 28, 2000	0	1	15	29	5
T. timopheevii	August 12, 2000	11	5	0	1	0
	August 28, 2000	0	2	8	5	2
T. turanicum	August 12, 2000	9	43	9	0	0
	August 28, 2000	2	2	18	15	24
T. turgidum	August 12, 2000	25	96	44	16	1
	August 28, 2000	13	10	63	86	56

[a] 1 = no recognizable infection; 2 = some florets infected on some plants; 3 = more than half the heads showing 25-50% infection; 4 = most heads showing 50% or more infection; 5 = all heads on a plant completely infected or dead.

Forty five hexaploid wheat introductions which had shown resistance in greenhouse tests were also evaluated. Four lines from CIMMYT (Fusarium BW # 3, 20, 21, and 25) and Chinese introduction 890037R rated 5% or less, indicating their value as resistant parents for crosses..

Table 3. Summary of FHB ratings for CDC Breeding lines tested at Carman in 2001

FHB INDEX[a]	No. Lines in each FHB Index Category - Carmen, 2001		
	HRS	DURUM	DH LINES
0 to 10	32	245	169
11 to 20	78	106	199
21 to 30	109	96	106
31 to 40	101	63	33
41 to 50	94	35	35
51 to 60	84	32	22
61 to 70	52	12	15
71 to 80	37	1	4
> 80	12	6	2
Total	599	596	585
Check Cultivar	**Mean FHB Index of Check Cultivars**		
FHB 37 (R)	6 ∀ 1.3	2 ∀ 1.0	8 ∀ 2.0
CDC Teal (S)	64 ∀ 3.9	45 ∀ 4.7	31 ∀ 3.3
Glenlea (S)	66 ∀ 2.1	33 ∀ 3.1	40 ∀ 5.0
AC Vista	20 ∀ 3.4	33 ∀ 3.3	23 ∀ 4.4
AC Morse	46 ∀ 4.6	19 ∀ 1.5	28 ∀ 3.6

[a] FHB Index = % head infection x % heads infected.

SPARC Breeding Material

Approximately 1,200 F4, F6 and F8 CWRS lines were grown. The distribution of FHB ratings for this material is shown in Table 4.

The FHB Index of the resistant checks (ND2710 and FHB #37) ranged 2-5 %, of the moderately resistant checks (China Scab # 37 and AC Barrie) ranged 10 -13 %, the moderately susceptible check (ND2827) 20%, and the susceptible checks (AC Morse, AC Vista, CDC Teal, Glenlea and AC Elsa) from 28 to 41%.

The number of lines with a FHB Index of 10 % or less (resistant) is greater in the F4 generation than the F8 generation and reflects emphasis that has been placed on using proven sources of FHB resistance and better selection techniques. F4 and F6 lines with an FHB index rating of 20% or

lower were multiplied in an off-season nursery for subsequent FHB testing in 2002. Twenty eight F8 lines were advanced to pre-registration trials.

Table 4. Distribution of FHB Ratings for SPARC Breeding Material tested at Carman in 2001

FHB Index	No. Lines in each FHB Index Category		
	F4 lines	F6 lines	F8 lines
0 to 10	181 (35 %)	111 (26 %)	14 (6 %)
11 to 20	78	151	44
21 to 30	88	80	35
31 to 40	73	53	41
41 to 50	58	24	31
51 to 60	23	11	29
61 to 70	15	0	9
71 to 80	1	0	0
> 80	1	0	0
Total	**518**	**430**	**219**

Other material included in this was 555 rows of F6 CWAD populations and 120 rows of an F6 CWES population tested. Of 27 families selected and increased in an off-season winter nursery, nine will be tested in 2002.

(b) **Minto Nursery**

CDC Breeding Material

Over 350 DH lines from eight CWRS cross populations were tested in the Minto nursery, most for the second time. Slightly over half (191 lines) will be retested. In addition, 986 F_3 lines from CWRS crosses were tested and 180 selected for greenhouse testing. A set of 22 potential parental lines were also included in this nursery. This testing confirmed the high level of resistance possessed by accessions, Fusarium BW #'s 3, 20 and 25. As well, Chinese lines J962413H and Wangshuibai also showed a high level of resistance.

Ten CPS F_2 bulk populations were grown. Approximately 200 heads were selected from each population and evaluated visually for shrunken seeds and "tombstone kernels". Approximately 10% of the lines from each population were retained for testing in 2002.

SPARC Breeding Material

Entries in the 2001 Minto nursery were:

(i) 648 bulk F4 CWRS populations of which 186 were selected and multiplied in the off-season nursery for testing in 2002;

(ii) 336 bulk F4 CPS populations of which 48 were selected and multiplied in the off-season nursery for testing in 2002; and

(ii) 1,379 single rows from F4 and F6 CWAD populations with either durum or novel sources of improved FHB resistance.

3. Year 2002 NURSERIES (Supported by ADF and WGRF funding)

(a) 2002 CARMAN NURSERY

CDC Breeding Lines

The development of the FHB infection in the 2002 nursery was poor and disease levels were low. However, sufficient differences were observed between the resistant and susceptible checks to identification of lines with good and poor levels of resistance, but discriminating among lines with intermediate disease ratings was not very effective. A summary of the FHB ratings for the CDC material tested in presented in Table 5.

Table 5. Distribution of FHB Ratings for CDC Breeding Material tested at Carman in 2002

FHB INDEX[a]	No. Lines in each FHB Index Category - Carmen, 2002		
	CWRS	DURUM	DH LINES
0 to 0.5	340	90	51
0.6 to 1.0	146	46	51
1.1 to 2	168	98	80
3 to 5	237	119	102
6 to 10	152	118	150
11 to 15	58	81	78
> 15	140	158	206
Total	**1241**	**710**	**718**
Check Cultivar	**Mean FHB Index of Check Cultivars**		
FHB 37 (R)	0.1 ∀ 0.0	1 ∀ 0.5	0.6 ∀ 0.1
ND2710 (R)	0.3 ∀ 0.1		1.2 ∀ 0.7
AC Cora	6 ∀ 0.7	9 ∀ 2.5	17 ∀ 2.5
AC Barrie (I)			17 ∀ 2.0

CDC Teal (S)	39 ∀ 3.4		21 ∀ 3.6
Glenlea (S)	21 ∀ 2.7	17 ∀ 4.0	21 ∀ 2.5
HY 446		17 ∀ 8.4	
AC Vista	18 ∀ 2.5	22 ∀ 3.8	28 ∀ 3.7
AC Morse	17 ∀ 2.5	21 ∀ 3.2	21 ∀ 2.4

[a] FHB Index = % head infection x % heads infected.

A group of 600 F5 lines selected on the basis of the 2001 Minto nursery FHB evaluations were grown in rows in the 2002 Carman Nursery. Of those 600 lines, 395 gave a resistant reaction. These lines were all advanced a generation in the greenhouse during the winter of 2002-2003 and were retested at Carman for FHB reaction and for rust reaction at Saskatoon in 2003. As well, 200 lines entered in the 2002 multi-location yield tests were also tested to gather FHB reaction data to assist in selecting those lines to be advanced into pre-registration trials.

Eight hundred, fifteen doubled haploid (DH) lines from 8 crosses were tested, many of them being retested to confirm their resistant reaction in 2001. Within these DH lines, a high frequency of resistance was found with 22% of the lines giving a FHB Index rating lower than 3. The average FHB Index of the checks was 1.2 for ND 2710 (resistant), 17 for AC Barrie (intermediate) and 21 for CDC Teal (susceptible). Lines with an FHB Index of less than 15 (approximately the AC Barrie level of resistance) were selected for retesting at Carmen and for rust reaction at Saskatoon in 2003.

A number of CWRS bulk populations were grown and random single-head selections made. Seed from these selections were evaluated during winter for FHB damage and the best retained for further FHB testing in 2003.

Eight CPS F_2 populations were evaluated for FHB resistance in short rows. Infection levels were quite variable, with FHB indices ranging from 0.5 to over 70. Many single plants with low infection scores were due to infection escape, largely due to late heading. Plants from those populations with low FHB indices were selected and increased in the greenhouse during winter.

Over 600 rows from 11 F_4 populations were also evaluated. These F_4 populations were derived from F_2 plants selected for moderate to high levels of FHB resistance at Minto in 2001. Their FHB indices ranged from a low of 0.1 to as high as 63, indicating considerable variation for FHB resistance. For the first time, 25 F_5 rows resulting from a cross between durum wheat and a moderately resistant spring wheat line were evaluated. Results from this cross are very promising. The FHB indices from this population ranged from 1 to 76.5. The average FHB index for AC Morse, the susceptible durum wheat check, was 48. All F_4 and F_5 populations at Carmen with an FHB index of 5 or less were identified, increased in the greenhouse, and will be evaluated again in 2003 at Carman.

Seven hundred and fifty lines displaying improved resistance to FHB in Carmen nurseries in 2001 were evaluated as F_5 hills in rust nurseries at Saskatoon in 2002. Five single heads were selected from all hills displaying acceptable agronomic traits and resistance to stem and leaf rust. These single heads will be grown as F_6 head rows in 2003 rust nurseries in Saskatoon.

AAFC-SPARC Breeding Lines

CWRS Populations

About 40% of the CWRS experimental lines expressed resistance comparable to the resistant checks. Lines with low FHB indices combined with acceptable agronomic performance, resistance to other diseases and end-use quality were increased in a winter-nursery. The most promising inbred lines and doubled haploid lines have been entered into pre-registration trials in 2003.

About 50% of the CPS experimental lines expressed resistance comparable to the resistant checks. These lines with low FHB scores combined with acceptable agronomic performance, resistance to other diseases and end-use quality were inbred in an out of season nursery. Nine promising inbred lines have been entered into pre-registration trials in 2003.

CWAD

756 F8 rows from F8 populations to transfer FHB resistance from novel sources were tested. The best lines identified were used in further crosses with adapted durum lines.

CWES

43 rows of an F8 population tested, and a line from the best family was selected for use in a further cross with a high quality, adapted parent.

Table 6. Distribution of FHB Ratings for AAFC-SPARC Breeding Material tested at Carman in 2002

Checks	FHB Index	FHB Rating	Rating Scheme
ND2710	0.2	R	R <=1
FHB#37	0.3	R	
China Scab #35	0.3	R	
Alsen	0.3	R	
AC Barrie	3.3	MR	MR 1.1 to 5
AC Cora	4.6	MR	
Glenlea	6	MS	MS 5.1 to 9
AC Morse	11	S	S > 9.1
AC Elsa	11	S	
AC Vista	18	S	
FHB index	**No. of experimental lines**		
	F4	**F6**	**F8**
0 to 1 = R	265	241	2
1.1 to 5 = MR	224	200	8
5.1 to 9 = MS	87	28	7
>9.1 = S	194	25	13
Total Lines	770	494	30

(b) 2002 MINTO NURSERY

CDC Breeding Material

Over 3,100 HRS wheat lines were evaluated for FHB reaction in the 2002 Minto nursery. Of these lines, 2,600 were F4 or F5 generation lines selected from 2001 hills rated as having some resistance to FHB. Approximately one-half of the 2001 selections had been retested for resistance in the greenhouse using the canary seed inoculation protocol during the winter of 2001-2002. Of the 2,600 lines tested in 2002, 600 were selected and increased in the greenhouse during the winter of 2002-2003. These lines are now being tested for rust reaction at Saskatoon in 2003 and for FHB reaction at Minto. The objective is to have the best of the lines in 2004 yield trials. A new group of populations selected from bulks grown at Carman in 2002 were grown in hill-plots testing in 2003.

A group of 500 lines with potential FHB resistance which were increased during the winter of 2001-2002 were grown in unreplicated yield trials at Saskatoon in 2002 and also tested for FHB reaction at Minto in 2002. From these 500 lines, 55 lines which combined FHB resistance with acceptable agronomic performance were selected, increased in the winter nursery and concurrently tested for quality. Only 15 lines survived this evaluation and are being yield tested at five sites in 2003 and retested for FHB reaction at Carman.

In 2002, over 500 doubled haploid lines from four populations were grown as hills at Minto, Manitoba and concurrently as hills in rust nurseries at Saskatoon. Those DH lines displaying improved FHB resistance, acceptable agronomic traits and resistance to prevalent races of stem and leaf rust were selected. Approximately 10% of the lines from each population were retained for unreplicated yield testing in 2003.

As part of the FHB breeding effort, all advanced breeding lines from the CPS program are evaluated for FHB resistance in hills at Minto. In 2002, approximately 200 lines with FHB resistance equal to or better than the moderately resistant checks were identified and advanced to replicated (if sufficient seed was available) or unreplicated yield trials in 2003.

AAFC-SPARC Breeding Lines

555 F6-derived F8 and 245 F2-derived F4 CWRS lines were evaluated for reaction to FHB at Minto, for stem and leaf rust at Winnipeg and for common bunt and agronomic performance at Swift Current. The most promising 105 F8 lines were submitted for quality analysis. The best 54 have been entered into the 2003 pre-registration trials. One doubled haploid line generated in 2000 with promising FHB resistance, resistance to other diseases, acceptable agronomic performance, and acceptable quality has been identified and advanced to pre-registration tests. The best 33 families are being yield tested at three locations in 2003. Thirty eight of the 245 F2-derived F4 lines were selected to be increased in a winter nursery for yield testing in 2003.

1,712 single rows from F4, F6 and F8 CWAD populations with adapted durum sources, bread wheat-derived sources, and *Triticum dicoccoides* sources of improved FHB resistance were also evaluated in the 2002 Minto nursery.

4. YEAR 2003 NURSERIES
(a) CARMAN NURSERY

CDC Breeding Material

In contrast to 2002, high levels of FHB occurred in the nursery. A summary of the FHB ratings of the lines tested is given in Table 7.

In 2003, six CPS F_2 populations are being evaluated for FHB resistance in F_2 short rows. Approximately 200-300 heads displaying improved FHB resistance will be selected from each population and evaluated visually for FHB-damaged kernels. About110 F_4 rows representing 10 CPS populations are also being evaluated plus 800 $F_{4:5}$ lines selected from the 2002 FHB nursery at Carman.

Approximately 40 CWAD $F_{4:6}$ lines are being evaluated for FHB resistance for the first time in 2003. These lines were derived using single seed descent from a cross between an elite breeding durum line and a hexaploid wheat with moderate levels of FHB resistance. Additionally, 25 F_6 durum rows from a second durum x moderately resistant spring wheat cross will be evaluated in 2003. These rows are derived from plants selected from F_5 rows at Carmen in 2002.

Table 6. Distribution of FHB Ratings for CDC Breeding Material tested at Carman in 2003

FHB Index[a]	No. Lines in each FHB Index Category - Carmen, 2003		
	CWRS	DURUM	DH LINES
0 to 10	94	196	223
11 to 20	150	200	169
21 to 30	229	181	166
31 to 40	129	102	67
41 to 50	123	95	39
51 to 60	105	70	26
61 to 70	60	40	12
> 70	64	102	20
Total	**954**	**986**	**722**
Check Cultivar	**Mean FHB Index of Check Cultivars**		
FHB 37 (R)	19 ∀ 3.4	11 ∀ 3.1	12 ∀ 2.5
ND2710 (R)			17 ∀ 3.3
AC Barrie (I)			20 ∀ 1.5
AC Cora (I)	36 ∀ 4.0	48 ∀ 5.6	32 ∀ 2.0
CDC Teal (S)	64 ∀ 1.6	62 ∀ 3.1	61 ∀ 2.4

AC Vista	73 V 2.7	69 V 2.6	69 V 2.6
AC Morse	50 V 3.6	55 V 3.2	63 V 3.0

[a] FHB Index = % head infection x % heads infected.

AAFC-SPARC Breeding Material
No report provided.

(b) MINTO NURSERY

CDC Breeding Material
In 2003, four CPS F_2 populations were evaluated for FHB resistance in bulk plots. Approximately 200-300 heads displaying improved FHB resistance were selected from each population and evaluated visually for FHB-damaged kernels. Over 200 CPS DH lines were evaluated for FHB resistance for the first time in 2003. Approximately 125 CPS lines with known sources of FHB resistance that have advanced to preliminary yield testing are also being evaluated for FHB resistance at Minto.

AAFC-SPARC Breeding Material
No report provided.

2. RESISTANCE SCREENING UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

The advantage of testing for disease resistance under controlled environmental conditions is that it provides the opportunity to reduce the variability association with disease evaluation under field conditions. In the case of FHB, it also provides the opportunity to test for different types of host resistance, specifically Type I (resistance to infection of the head) and Type II (resistance to fungal spread within the head) resistance.

Two inoculation methods have been used: spraying heads soon after emergence with a spore suspension of *Fusarium graminearum* and inoculating heads by placing a FHB inoculum in a central floret 2-3 days after anthesis. The first method evaluates both Type I and Type II resistance, whereas the second evaluates Type II resistance only. However, preliminary studies indicated that floret inoculation produced more repeatable results than spray inoculation, and since Type II resistance is thought to be more closely associated with mycotoxin production, this method was chosen as the primary method of inoculation in controlled environmental testing. This choice led to studies to determine the best inoculation method and disease rating method to use.

OBJECTIVES:

The overall objective was to determine the FHB reaction of resistance sources and selected advanced breeding lines under controlled environmental conditions.
Within this objective, studies were also conducted at the Crop Development Centre, University of Saskatchewan, to:

(a) To compare two floret inoculation methods, inoculation with a conidial suspension and inoculation with infected canary seed, for differences in their ability to identify Type II resistance in a range of wheat cultivars possessing different levels of resistance
(b) To compare two methods of disease evaluation, a single rating based on percentage area of the head infected and the area under the disease progress curve (AUDPC), on a set of genotypes representing a range of resistance levels.
(c) To examine the pathogenicity of four *Fusarium* species on wheat cultivars with different levels of resistance and to determine if host resistance to *F. graminearum* is also effective against other *Fusarium* species

1. COMPARISON OF TWO FLORET INOCULATION METHODS FOR DETERMINING TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT

Materials and Methods

The spring wheat cultivars used were MN 2540 (a moderately resistant line from the University of Minnesota, designated as MN), AC Barrie (intermediate) and CDC Teal (susceptible). These were tested with a F. graminearum culture isolated from infected seed from Saskatchewan in 2000 using two inoculation methods, infected canaryseed and conidial suspension at concentrations of 5,000 spores/mL and 10,000 spores/mL.

The canary seed inoculum was prepared using a modification of the method of Jin et al.(1999). *F.graminearum* isolated in Saskatchewan grown on cornmeal agar for 5-7 days. Cleaned canary seed was soaked in distilled water for 24 h, spread in a thin layer in a glass petri dish and autoclaved. The sterilized canary seed in each dish was inoculated with pieces of agar cut from a *F. graminearum* culture grown on cornmeal agar for 5-7 days and cultured for 10-12 days at room temperature under continuous fluorescent light. The colonized seeds were air dried and stored at 4°C until use. The conidial suspensions were prepared from a 5-7 day old F.graminearum culture grown on corn meal agar. The conidia were gently washed with distilled water from the surface of the culture into a glass flask and spore suspension adjusted to appropriate concentration using a hemacytometer and stored at 4° C. A fresh conidial suspension prepared every two days

Heads of each wheat cultivar were inoculated when the anthers were beginning to extrude from the central florets, Infected canary seed was soaked in distilled water for 20 minutes and one seed was placed in the primary floret of a spikelet in the centre of the head. For conidial inoculation, 10 µl of conidial suspension were injected into a floret of the centre spikelet using a micropipettor. One head of each plant in the pot was inoculated (3 heads/pot). After inoculation, plants were placed in a moist chamber for 24 h. The infected heads were rated for disease severity at 7, 14 and 21 days after inoculation using the following rating scale of.

RATING SCALE for FHB POINT INOCULATIONS (Murphy et al.1990)

0 = no infection
1 = only inoculated floret infected
2 = only inoculated spikelet infected
3.0 = inoculated spikelet and rachis infected
3.2 = inoculated spikelet and one adjacent spikelet infected
3.4 = inoculated spikelet and two adjacent spikelets infected
3.6 = inoculated spikelet and three adjacent spikelets infected

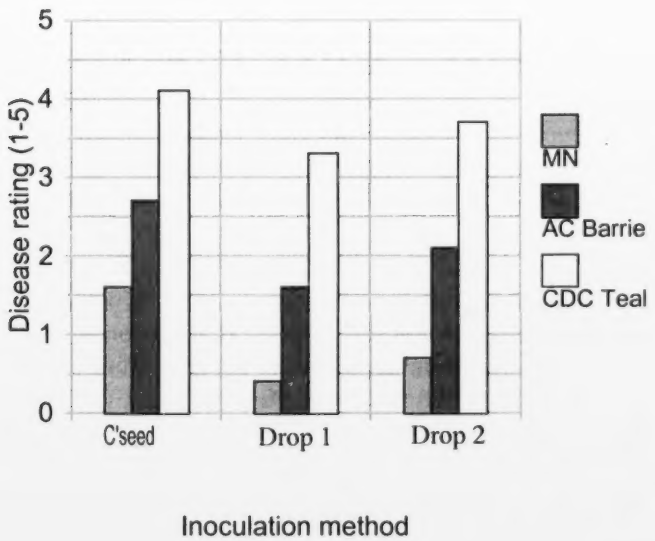
3.8 = inoculated spikelet and four adjacent spikelets infected
4 = half spike infected
5 = whole spike infected
On this scale, we considered 0,1 = Highly Resistant; 2,3.0 = Resistant; 3.2-3.4 = Moderately Resistant; 3.6-3.8 = Moderately Susceptible; and 4+ = Susceptible.

RESULTS

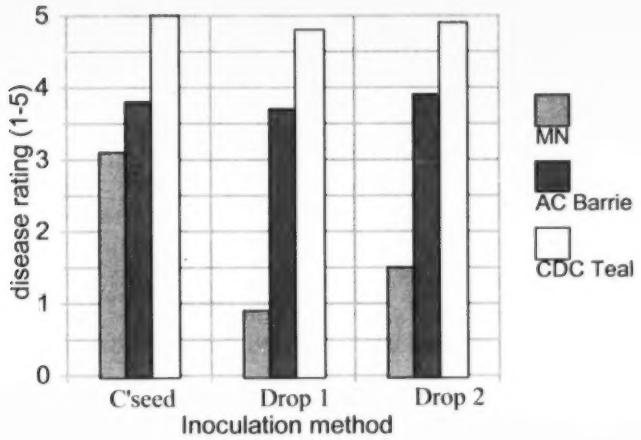
Analysis of Variance for Disease Rating

Days after Inoculation		7 days	14 days	21 days
Source	DF	MS	MS	MS
Cultivar	2	36.13**	43.52**	35.76**
Inoculation Method	2	5.09**	2.93**	2.20**
Cv*Inoc. Method	4	0.11	2.26**	2.31**
Error	45	0.53	0.31	0.33

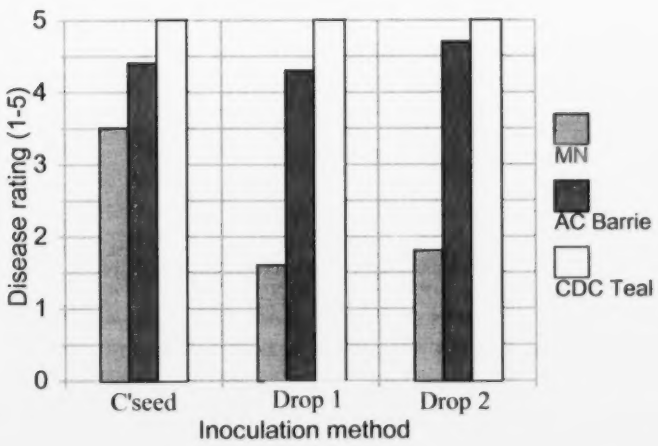
FHB ratings at 7 days



FHB ratings at 14 days



FHB ratings at 21 days



Testing at the Crop Development Centre

CWRS Breeding Lines
Over 1,000 new DH lines were evaluated for FHB reaction in greenhouse tests using the canaryseed inoculation protocol during the winter of 2000-2001. The lines were concurrently increased in a winter nursery and are currently being field-tested. These lines represent a further round of backcrossing to well-adapted CWRS parents.

Identification of Resistance Sources
200 tetraploid germplasm accessions were screened for FHB resistance during the winter of 2000 using both the infected canaryseed inoculation procedure and by spraying the entire spike with a conidial suspension of Fusarium. These accessions were also tested at Minto in 2000. Those accessions rated resistant in the field nursery were retested in the greenhouse during the winter of 2001 using only the canaryseed inoculation method. Of the germplasm screened to date, 17 lines that gave low FHB scores in two greenhouse tests and one field test have been identified. These lines will be considered as possible parents and will be tested further to confirm their value as sources of resistance.

Eighty-five common wheat lines were tested using infected canaryseed inoculation in a three-replicate test to identify new sources of resistance. This material included introductions from China, Japan and CIMMYT, Mexico. Almost one-third (26 lines) were rated highly resistant. While much of this material likely derives its resistance from the widely-used resistance source Sumai 3, at least five lines are potentially new resistance sources as judged from their pedigree and/or origin. Three of the highly resistant lines are introductions from Japan. Also among this material are four DH lines developed from crosses between CDC Teal and two Chinese lines whose resistance is thought to be different to Sumai 3.

Sixty-seven spring wheat accessions from 13 countries have recently been received for greenhouse testing. These represent the best of the sources of resistance tested in 1998 and 1999 as part of the U.S. Wheat and Barley Scab Initiative.

Testing at AAFC - SPARC
Approximately 75 common wheat and 150 durum wheat lines were tested in greenhouse/growth room tests in 2000-2001.

2. RESISTANCE SCREENING UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

Comparison of Inoculation Methods for Testing Type 2 Resistance to Fusarium Head Blight
We have used two inoculation methods in greenhouse tests screening for Type 2 resistance (resistance to fungal spread within the head). One method involves placing a measured drop of *Fusarium graminearum* spore suspension in a central floret of a wheat head with a micropipette (drop inoculation), the other involves placing a canary seed infected with *F. graminearum* in a central floret. The second method is faster and less technically demanding than the first, which is the accepted standard method of inoculation.

This study was conducted to compare the ability of these two inoculation methods to identify resistance in three wheat cultivars with different levels of Type 2 resistance, MN2540 (R), AC Barrie (I) and CDC Teal (S) using a single isolate of *F. graminearum* from Saskatchewan. Resistance expression was rated at 7, 14 and 21 days after inoculation. Results from a single replicated experiment showed that (1) inoculation method had no effect on cultivar ranking for resistance; (2) that infected canary seed inoculation induced higher levels of disease than drop inoculation; and (3) drop inoculation using a spore suspension at one-half the standard spore concentration was as effective as the standard spore concentration.
Based on these results, it was concluded that because of ease of inoculum preparation and inoculation procedure, canary seed inoculation is the preferred method for routine screening, particularly if high levels of resistance are to be identified. However, drop inoculation should be used when more precise results are desired and when evaluating for small differences in resistance. Such differences are likely to be masked by the higher disease levels obtained when canary seed inoculation is used.

TESTING AT THE CROP DEVELOPMENT CENTRE

METHODOLOGY

Inoculation with infected canaryseed is the routine inoculation methods used in greenhouse screening for FHB resistance. A single infected seed is placed in a central floret 2-3 days after anthesis and the inoculated p[plants are then placed in a moisture chamber for 24 h. Rating for FHB resistance, based on extent of head infection, is done 19-21 days later.

RESULTS

CWRS Breeding Lines

One hundred and eighty F4 lines identified in the 2001 Minto nursery were re-tested in the greenhouse during the winter of 2001-2002. Of these, 85 lines were identified as resistant. These 85 lines have now been challenged with FHB for three consecutive generations and it is our hope that they have a high level of resistance to the pathogen. Another group of lines selected from bulks grown at Carman in 2001 were also evaluated in the greenhouse. A series of 268 new DH's were also screened for FHB reaction.

Identification of Resistance Sources

Sixty-seven spring wheat accessions from 13 countries, representing the best of the sources of resistance tested in 1998 and 1999 as part of the U.S. Wheat and Barley Scab Initiative, were tested. Thirty one were retained for retesting and of these, resistance was confirmed for 12 lines from Brazil, China, Japan, and Italy. A listing of these accessions follows:

Shirasagi Komugi (PI 360870)
Fujimi Komugi (PI 360869)
533B (PI 349534)
Norin 34 (PI 182568)
Norin 20 (PI 155265)
Sichuan 9418 (PI 559646)
Nobeoka Bozu (PI 382153)
Funo (A) (PI 213833)
Shu Chou Wheat No. 3 (PI 46215)
Tokai 66 (PI 382161)
Buck Austral (PI 344454)
Gogatsu Komugi (PI 411132)

OTHER STUDIES AT CDC

Pathogenicity of Fusarium Species Associated with Fusarium Head Blight of Wheat (FHB)
A number of Fusarium species have been isolated from wheat heads showing symptoms of FHB in Saskatchewan, however it is not known whether all are equally pathogenic and whether the same host resistance is effective against them all. This study was performed to try to answer those questions. Four wheat cultivars with different levels of Type 2 resistance, ND2710 (R), Wangshuibai (MR), AC Barrie (I) and CDC Teal (S), were inoculated with a Saskatchewan isolate of each of *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. This replicated test was conducted in the greenhouse using the infected canary seed method of inoculation of one central floret per head per plant. Disease rating was commenced four days after inoculation and continued at two-day intervals until 22-24 days after inoculation or until the head was completely infected.
The disease progression data were used to calculate standardized AUDPC values for each cultivar to account for differences in rating period. Both *F. graminearum* and *F. culmorum* were highly pathogenic and produced similar disease responses on each cultivar, whereas *F. avenaceum* and *F. poae* were only weakly pathogenic and induced little to no disease. Host resistance to *F. graminearum* appeared to be effective also against *F. culmorum*.
These preliminary results suggest that although many Fusarium species may be involved with FHB, only certain species are of importance in terms of breeding for resistance; these also are the most important toxin-producing species. As well, it appears that a different resistance is not needed for each pathogenic species. Thus disease surveys to monitor the relative frequency of the different *Fusarium* species are important to assess the threat of FHB in Saskatchewan. Since *F. avenaceum* is commonly isolated in Saskatchewan, its weak pathogenicity, as found in this study, needs to be confirmed by additional studies using a larger array of isolates.

Testing at AAFC – SPARC
Lines identified as resistant in a FHB nursery at Portage la Prairie, Manitoba were progeny tested by single floret inoculation with a conidial suspension of *F. graminearum*. The most resistant types were also have been subjected to molecular marker analysis. Three replications of five sub selections of three CPS, three CWRS and one Hard White line were used. The information gathered has been used to design new crosses for molecular marker-assisted selection for FHB resistance. About 75 other potentially resistant hexaploid wheat lines were also screened.

2. RESISTANCE SCREENING UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

AAFC - SPARC (Supported by ADF and AAFC funding)
Lines with putative FHB resistance tested at a nursery at Portage La Prairie were progeny tested by injecting a conidial suspension of FHB into the wheat florets. Also, the most resistant types have been analyzed using DNA molecular markers for FHB resistance. Three replications of five sub-selections of three CPS, three CWRS and one Hard White lines were used. The information has been used to design new genetic crosses so that DNA molecular markers can be applied.

CROP DEVELOPMENT CENTRE

Breeding lines evaluated in the greenhouse using the canary seed inoculation method was described with the results from the 2002 field nurseries.

OTHER STUDIES AT CDC

Pathogenicity of Fusarium Species Associated with Fusarium Head Blight of Wheat (FHB)

The study conducted in 2001-2002 to compare the pathogenicity of Fusarium species isolated from wheat heads showing symptoms of FHB was repeated in 2002-2003 using isolates from Manitoba and Alberta and including an additional *Fusarium* species, *F. sporotrichoides*. The same four wheat cultivars with different levels of Type 2 resistance were used, ND2710 (R), Wangshuibai (R), AC Barrie (I) and CDC Teal (S), and were inoculated with an isolate from each of Alberta, Manitoba and Saskatchewan of each of *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *F. sporotrichoides*. This replicated test was conducted in the greenhouse using the infected canary seed method of inoculation of one central floret per head per plant. Disease rating was commenced four days after inoculation and continued at two-day intervals until 22-24 days after inoculation or until the head was completely infected.

The disease progression data were used to calculate standardized AUDPC values for each cultivar to account for differences in rating period before statistical analysis was performed. The results obtained confirmed the findings of the first experiment. Both F. graminearum and F. culmorum were highly pathogenic, whereas *F. avenaceum*, *F. poae* and *F. sporotrichoides* were weakly pathogenic and induced little disease. Host genotypes ND2710 and Wangshuibai had the lowest AUDPC values, AC Barrie was intermediate and CDC Teal the highest value, confirming the resistance of the first two host genotypes. No differences among isolates in terms of their origin were found, nor was there a species x location interaction.

A significant host genotype x species interaction was obtained, indicating the reaction of host genotypes to each *Fusarium* species was not always the same. This interaction appeared due to the host genotypes showing greater differences in FHB development when infected with *F. graminearum* than when infected with *F. culmorum*. However, the relative resistance ranking of host genotypes remained the same. This result confirmed that, for these host genotypes, selection for resistance to *F. graminearum* also selected for resistance to *F. culmorum*. It also suggests that testing for resistance to *F. graminearum* is likely to identify greater differences in resistance levels than if *F. culmorum* was used.

These results continue to suggest that although many *Fusarium* species may be involved with FHB, only certain species are of importance in terms of breeding for resistance. This study will continue using a more geographically diverse collection of isolates to test the preliminary finding that geographic origin of isolates has no impact on pathogenicity.

4. MOLECULAR MARKER-ASSISTED SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE

INTRODUCTION

Breeding for FHB resistance is complicated by factors such as agronomic undesirability and poor quality attributes of resistant germplasm, quantitative inheritance of resistance and the large role the environment plays in disease development and expression of resistance. Use of molecular markers to indirectly select for FHB resistance genes can be used to increase selection efficiency in breeding by removing the interference by the environment on line selection. Chromosomes 3BS, 5AS, 6AS, and 6BS have been reported to carry FHB resistance genes in Sumai-3 and its derivatives, with the quantitative trait locus (QTL) Qfhs.ndsu-3BS of major effect being located on chromosome 3BS. Single sequence repeat (SSR) markers Xgwm493 and Xgwm533 were significantly associated with FHB resistance and are believed to flank this QTL A FHB-MAS method outlined in Anderson et al. (2001) is available (see http://maswheat.ucdavis.edu/protocols/FHB/index.htm, but it is not suitable for screening large numbers of breeding lines in a laboratory with only basic equipment for molecular work. A simplified method for screening for Qfhs.ndsu-3BS will be of interest to wheat breeders, particularly if only a small investment in equipment (e.g. thermocycler, centrifuge, and two gel electrophoresis units) is required.

This portion of the project had the following objectives:

(a) to develop a simplified FHB-Marker-Assisted selection procedure using molecular markers Xgwm493 and Xgwm533, associated with Sumai-3 resistance; and
(b) to apply this protocol to identify breeding material and germplasm carrying the Sumai 3-type resistance

MATERIAL AND METHODS

Seven common and one durum wheat were used in this study. Their origin, pedigree and reaction to FHB are shown in Table 1.

Approximately 51 FHB susceptible parental genotypes, named cultivars or advanced breeding material from Canadian programs, were screened using multiplexed PCR reactions prior to initiating the screening of over 5,500 breeding lines for the 3BS FHB QTL (data not shown). All susceptible genotypes were determined to be distinctive enough in amplicon size relative to the resistant parents for the screening of populations to proceed using 2% agarose gel electrophoresis. Routine screening within crosses for the HY644 or Alsen resistant parental phenotypes were done using non-multiplexed PCR reactions (i.e. using Xgwm533 only).

Seedling Germination

DNA template was obtained from seeds germinated as described by Eckstein et al. (In review) with the following adaptations. Three seeds were germinated per plug. Tissue was generally harvested from 5-day old seedlings, but up to 14-day old tissue could be used for DNA extraction, thus allowing for flexibility within the procedure. Seeds were germinated in plug trays for 5 days in darkness at 23°C and, if necessary, transferred to 24 h light (50 µmol $m^{-2}s^{-1}$). Light was provided by four 34 W cool-white fluorescent lamps mounted horizontally above the trays. Seedlings were fertilized weekly

with a dilute solution of soluble 20-20-20 All Purpose fertilizer (Plant Products Co. Ltd.; Brampton, ON). For controls, seed of Sumai-3 along with the resistant and susceptible parents of the cross of interest were placed in the first three tray cells, with an additional Sumai-3 control placed at random within the rest of the tray. The Sumai-3 controls were used to ensure that the correct orientation of the 96-well microplate was maintained throughout the procedure. Sumai-3 was chosen as it, unlike most of its other derivatives (e.g., ND2710, McVey, HY644, Alsen), possessed an additional 100 bp amplicon identified using primer Xgwm533.

Table 1. Origin, pedigree, Fusarium head blight (FHB) reaction, and amplicon size (bp) produced using Xgwm493 and Xgwm533 primers for the eight wheat genotypes used in this study.

Line	Source	Pedigree	FHB reaction †	Xgwm533 (bp)	Xgwm493 (bp)
Sumai-3	China	Funo/Taiwan wheat	Resistant	160	213
ND2710	USA	ND2603/Grandin	Resistant	160	213
McVey	USA	Ning 8331/MN87029//MN89068	Resistant	ND	ND
HY644	Canada	A16//Alpha*4/BgBSR/3/Sceptre/Ning 8331	Resistant	160	157
Glenlea	Canada	Pembina*2/Bage//CB100	Susceptible	133	211
Superb	Canada	Grandin*2/AC Domain	Susceptible	ND	157
AC Foremost	Canada	HY320*5/BW553//HY320*6/7424-BW5B4	Susceptible	133	177
Commander	Canada	W9260-BK03/AC Navigator//AC Pathfinder	Susceptible	ND	ND

† For Sumai-3, ND2710, HY644, Glenlea, Superb, and AC Foremost, the FHB reactions and amplicon sizes were characterized by McCartney et al. (2004). The FHB reaction for cv. McVey was described by Busch et al., 2001 and for cvs. Superb and Commander by Saskatchewan Agriculture, Food, and Rural Revitalization (2005). ND, Not determined.

DNA Extraction

DNA was extracted from 5- to 14-day old seedling tissue as described by Eckstein et al. (In review) with the following adaptations. Tissue was harvested from the upper portion of the coleoptile by removing a 1.5 cm-l piece with tweezers, macerated lengthwise using tweezers and then transferred into the well bottom of a non-skirted 96-well microplate (VWR International Cat No. 82006-636; Mississauga, ON). [NOTE: This step can be non-destructive if the remnant portions of the seedlings are placed under continuous light for 7 days and then planted in pots.] The tweezers are washed in 95% ethanol after each harvest. The microplate was centrifuged at 2755 x g for 1 min to ensure the macerated tissue is at the bottom of the wells. The amount of plant tissue is critical to ensure consistent results. The microplate does not have to be placed on ice at any point during harvesting or DNA extraction.
After centrification, 40 µL of 0.25 M sodium hydroxide is added to each well and the uncovered microplate is heated at 94°C for 2 min using a pre-heated 96-well thermal cycler block. The plate was removed from the thermal cycler, set at room temperature, and 60 µL 0.5 M Tris-HCL pH 8.0 added immediately to each well. The uncovered microplate was heated again at 94°C for 6 min and then centrifuged at 2755 x g for 2 min. If to be used the same day, the microplates containing the DNA template can be stored in a sealed plastic bag at 4°C, until needed. For longer term storage, the

microplates were covered aluminum foil and stored in a sealed plastic bag at –20C. The microplates can be frozen for up to 30 days without significant loss of amplification quality.

PCR Amplification

Reactions were performed in a fresh 96-well microplate by adding one drop of light mineral oil (BDH Inc., Toronto, ON; Cat. No. K91960) to each well and transferring 2 uL of DNA extract using a 12 channel x 20 µL multichannel pipette by piercing, if needed, through the aluminium foil. The microplate was centrifuged at 2755 x g for 1 min to prevent the DNA template from evaporating from the wells and then stored at room temperature until needed. The remnant DNA template can be refrozen in case a re-run is desired.

Two primer sets were used in the PCR screening, Xgwm493-3B (forward primer 5' TTCCCATAACTAAAACCGCG 3' and reverse primer 5' GGAACATCATTTCTGGACTTTG 3'), and Xgwm533-3B (forward primer 5' AAGGCGAATCAAACGGAATA 3' and reverse primer 5' GTTGCTTTAGGGGAAAAGCC 3'). All reactions were performed on a PTC-100 MJ Research DNA Thermal Cycler with 2 ml of DNA extract amplified in a 20 ml reaction volume. For multiplexed PCR reactions, the mixture consisted of 1X PCR buffer, 50 mM $MgCl_2$, 0.15 mM of each Xgwm 493-3B primer (Invitrogen, Carlsbad, CA), 0.10 mM of each Xgwm 533-3B primer (Invitrogen), 200 mM of each dNTP (Invitrogen; Cat No. 10297-018), and 0.5 U Taq DNA polymerase (Invitrogen, Carlsbad, CA; Cat No. 18038-042). For non-multiplexed PCR reactions, the mixture was the same as for multiplexed reactions, except that 0.10 mM of each primer was used. For ease,1,800 µL master PCR mixture was prepared in a 5- mL tube. After 18 µL of the master PCR mixture was added to each well, the microplate was centrifuged at 2755 x g for 1 min. For both the multiplexed and non-multiplexed reactions, the PCR amplification conditions were identical and included an initial 3 min denaturing step at 94°C followed by 38 cycles of denaturing (94°C) for 30 sec, primer annealing (52°C) for 40 sec, extension (72°C) for 55 sec, and a final extension of 3 min at 72°C. If needed, reactions were held at 10°C overnight.

Electrophoresis

Products from the PCR amplification reactions were separated by electrophoresis at 155 V for 75 min on a 150 mL 1X TBE (45 mM Tris-borate, 1 mM EDTA), 2 % (w/v) UltraPure agarose gel 027) containing 2 µL ethidium bromide. Microplates were centrifuged at 2755 x g for 1 min after adding 4 µL of loading buffer (0.25% bromophenol blue, 40% [w/v] sucrose) to each well. Approximately 10 µL from each reaction, containing loading buffer, was loaded per lane and visualized with UV light. The remnant portion of the PCR reaction was stored at –20°C in a sealed plastic bag in case a re-run was desired.

Agarose gel analysis was conducted using a Sunrise 96 Biometra horizontal gel electrophoresis apparatus that allowed for the simultaneous evaluation of 104 samples. Approximately 0.5 µg of 1 Kb Plus DNA ladder (Invitrogen; Cat. No. 10787-018), suitable for sizing DNA fragments from 100 bp to 12 Kb, was loaded into the first and last lane of each of the four combs. A 12-channel x 20 µL multichannel pipette was used to load gels with samples A1-12 and B1-12 loaded into the first 24 wells (first comb) and so on.

Samples within the gels were scored as possessing a resistant or susceptible phenotype relative to the parental controls run concurrently within each gel. Any sample possessing a phenotype different to the resistant parental phenotype (i.e., segregants) were classed as susceptible.

RESULTS

The amplicon size using primers Xgwm533 and Xgwm493 are given for Sumai-3, ND2710, McVey, HY644, Glenlea, Superb, AC Foremost, and Commander in Table 1. Amplification products observed using Xgwm533 and Xgwm493 in FHB resistant (Sumai-3, ND2710, McVey, HY644) and susceptible (Glenlea, Superb, Foremost, and Commander) genotypes were separated on ethidium stained 2% agarose gels (Fig. 1).

Amplification products are presented for non-multiplexed and multiplexed PCR reactions (Fig. 1). Amplicon sizes for the genotypes tested were between 220 and 100 bp. The phenotype for Sumai-3 and ND2710 were identical, except for an additional 100 bp fragment amplified by primer Xgwm533 in Sumai-3. The phenotype for Alsen (data not shown) using multiplexed PCR reactions was identical to that of HY644. Separation of the 157 and 160 bp fragments in HY644 was not possible using 2% agarose gel electrophoresis. However, when primer Xgwm533 was used, an amplicon mobility difference of ~30 bp was observed between the four susceptible genotypes (~133 bp) and HY644 (160 bp) and was sufficient for visualization using 2% agarose gel.

The phenotype for McVey was similar to Sumai-3 when primer Xgwm493 was used, but it carried a susceptible allele (~133 bp) which was identified by primer Xgwm533. The phenotypes of BacUp (data not shown) and Glenlea were identical to McVey. The phenotypes of the susceptible genotypes were similar to Glenlea when primer Xgwm533 was used were all similar to Glenlea (~133 bp) while the amplicons observed using primer Xgwm493 were at least 36 bp smaller than for Sumai-3.

Approximately 3,900 Canada Western Red Spring (CWRS) wheat breeding lines were screened and 1,225 lines (33%) possessed the resistant parental phenotype (Table 2). Ten different doubled haploid populations involving ND2710, McVey, BacUp, or Sumai-3 as the FHB resistant parent were screened using multiplexed PCR. Of the 411 lines screened, 6% possessed the resistant parental phenotype. The F_3 and F_4 generations of 13 crosses involving Alsen as the resistance source were screened using primer Xgwm533. Of 3,258 lines screened, about 35% possessed the Alsen resistance phenotype with the frequency of resistant lines in the individual populations varying from 26 to 44%. F_7 lines in advanced testing (lines from 2004 AGWYT 1 to 11) from 20 different crosses involving resistant parents Alsen, ND2710, McVey, or BacUp were screened using multiplexed PCR.. Of the 238 lines screened, approximately 22% resembled the appropriate resistant parental phenotype.

Approximately 1,660 Canada Prairie Spring (CPS) wheat breeding lines were screened and 596 lines (36%) possessed the resistant parental phenotype (Table 2). F_3 or F_4 generations of four crosses involving resistant parents HY644 or ND2710 were screened using non-multiplexed (to detect HY644-type resistance) or multiplexed PCR (to detect ND2710-type resistance). Of the 905 lines screened 36% showed a resistant phenotype. In addition, F_5 populations of four crosses involving either HY644 or ND2710, F_6 of seven crosses involving HY644 or McVey, and F_6 lines, from five crosses involving ND2710, McVey, BacUp or HY644, in early generation testing were screened with either non-multiplexed or multiplexed PCR . The frequency of resistant genotypes identified is shown in Table 2.

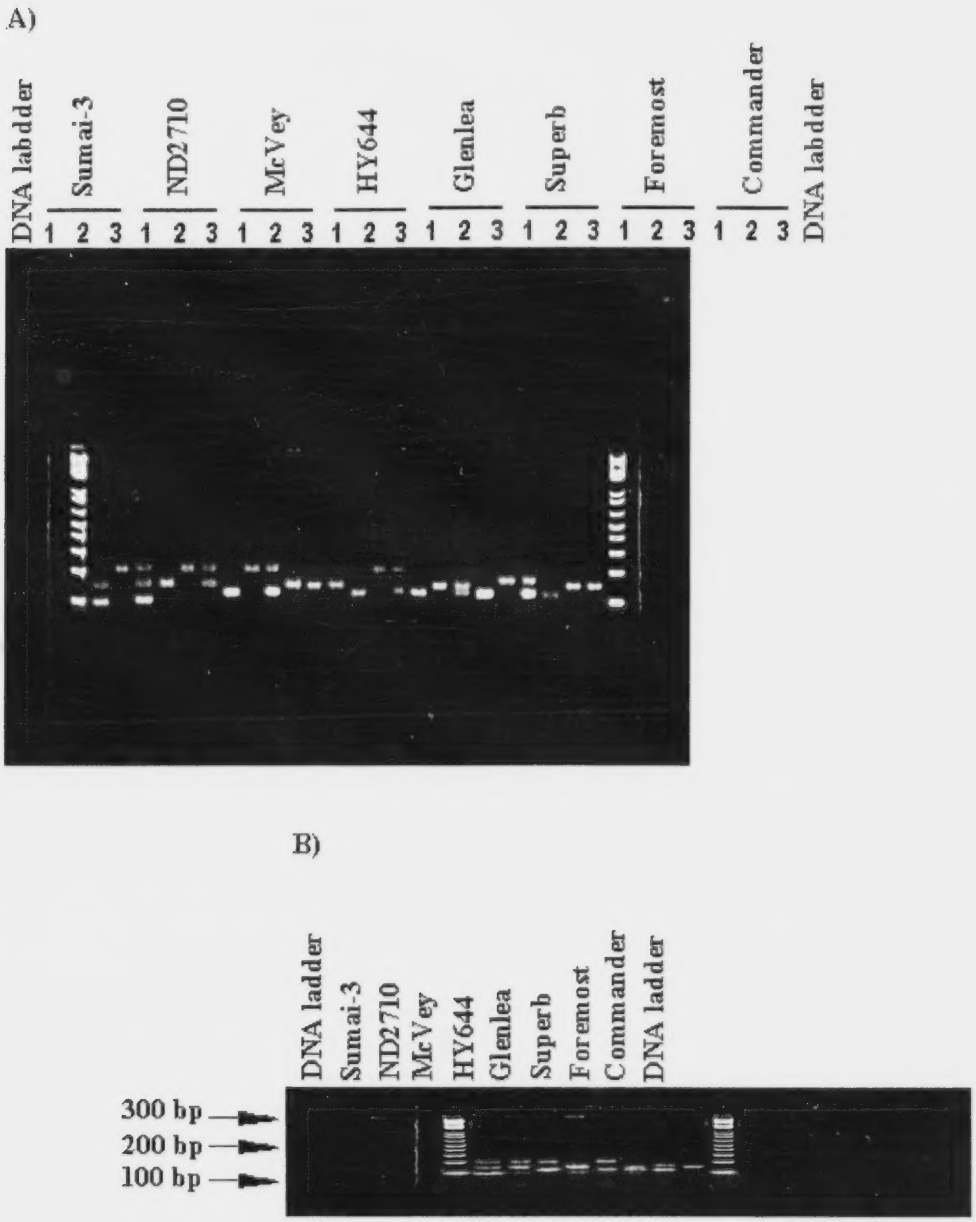


Fig. 1: Amplification products, using wheat microsatellite primers *Xgwm*533 and *Xgwm493*, observed in FHB resistant (Sumai-3, ND2710, McVey, HY644) and susceptible (Glenlea, Superb, Foremost, Commander) genotypes and separated on ethidium stained 2% agarose gels. In (A) amplification products for the eight genotypes are presented for PCR reactions derived using primer *Xgwm*533 only (lanes 1), primer *Xgwm493* only (lanes 2), and both primers *Xgwm*533 and *Xgwm493* (lanes 3). In (B) amplification products for the eight genotypes are presented for multiplexed PCR reactions only to compare amplicons derived in FHB resistant vs. susceptible genotypes. In (A) and (B), the first and last lanes illustrate the 100, 200, and 300 bp fragments observed for the 1 Kb Plus DNA ladder.

Table 2. Wheat class, pedigree, generation, number of lines screened for the 3BS FHB QTL using primers *Xgwm*493 and *Xgwm*533, and number of lines with resistant parental phenotype detected for breeding lines screened within this study.

Wheat class	Pedigree	Generation	No. of lines screened	No. of lines with resistant parental phenotype	
CWRS	BW693/BW278 †	DH	23	0	(0%)
	BW277/BW279 †	DH	59	0	(0%)
	Teal/FHB1 ‡	DH	8	3	(38%)
	Teal/FHB2	DH	57	17	(30%)
	Teal/FHB3	DH	78	2	(3%)
	Teal/FHB5	DH	49	0	(0%)
	Teal/FHB9	DH	34	0	(0%)
	BW720/FHB3	DH	14	1	(7%)
	BW720/FHB8	DH	18	1	(6%)
	BW720/FHB9	DH	64	0	(0%)
	2000 Carmen DH hills (<10% FHB)	DH	7	4	(60%)
	Alsen//BW799/W00174	F3	380	152	(40%)
	Alsen//BW314/W99289	F3	380	156	(41%)
	Alsen/P00.06-33	F3	67	28	(42%)
	Alsen/P00.06-78	F3	273	112	(41%)
	Alsen//W01566	F3	380	167	(44%)
	Alsen/P00.02-56	F4	356	93	(26%)
	Alsen/P00.05-86	F4	374	4	(1%)
	Alsen/P00.38-39	F4	356	153	(43%)
	Alsen/P99.51-142	F4	380	148	(39%)
	Alsen/P99.51-73	F4	312	131	(42%)
	2004 AGWYT1 to 11	∃F7	192	42	(22%)
	2001 FHBYT	∃F7	46	11	(24%)
		TOTAL	**3907**	**1225**	**(31%)**
CPS	HY644/HY459	F3	226	79	(35%)
	HY644/HY962	F3	239	88	(37%)
	98W1147/HY962	F3	180	61	(34%)
	98MDG147/98W1147	F4	260	96	(37%)
	U97016/HY644//98W1147	F5	52	22	(42%)
	HY528/98W1147	F5	23	15	(65%)
	HY528/HY644	F5	146	44	(30%)
	HY962/HY644	F5	236	71	(30%)
	HY962/BW278//98W412	F6	26	25	(96%)
	HY962/BW279//98W412	F6	16	2	(13%)
	HY962/94C15*A07//HY644	F6	35	23	(66%)
	HY962/94C15*G44//HY644	F6	34	13	(38%)
	HY459/97W488	F6	6	1	(17%)
	HY644/HY962	F6	105	3	(3%)
	2004 EGHYT 1 to 8	F6	76	53	(70%)
		TOTAL	**1660**	**596**	**(36%)**

† BW278 and BW279 have a susceptible phenotype with primers 533 and 493 but they still carry the QTL for FHB. Thus our FHB-MAS method cannot identify resistant phenotypes in crosses involving these two resistant parents.

‡ FHB1, FHB2, & FHB3= Teal/ND2710/Teal; FHB5= Teal/BacUp//Teal/McVey, and FHB8 & FHB9= Teal/ND2710//Teal/McVey.

DISCUSSION

The FHB-MAS method outlined in this study has been adapted from the original method reported by Anderson et al. (2001) and provides an inexpensive means ($2 per sample) for routine screening of 1000 breeding lines per person per 40 h week.. The adaptations made include 1) using a 96-well DNA extraction procedure, 2) reducing the annealing temperature from 60°C to 52°C, 3) reducing the PCR cycle duration from 3 1/2 h to 2 h, 4) multiplexing PCR reactions to simultaneously screen for *Xgwm493* and *Xgwm533*, and 5) replacing the use of sequencing gels, requiring silver staining, with 2% agarose gels stained with ethidium bromide. This simple, rapid, and inexpensive PCR-based method has particular value in applying marker-assisted selection for the major FHB gene on chromosome 3BS to both early and advanced generation breeding lines..

Breeding populations developed with parents carrying the 3BS QTL can be assessed with markers mapping near the QTL, but routine field-based FHB resistance phenotyping in disease nurseries should be carried out as well. This approach makes use of previous mapping information and has the potential to rapidly develop adapted Canadian cvs. which carry at least one major FHB gene. Using the FHB-MAS method outlined in this study to screen 5,567 breeding lines from CDC wheat breeding programs showed unacceptable level of correspondence between field-based FHB resistance ratings and the results of the marker screening. It is expected that selecting for the 3BS QTL in early generation lines (F_6) together with field FHB testing will result in a higher frequency of advanced lines with Type II resistance to FHB.

The most common source of the FHB resistance being used in Canadian breeding programs is derived from Chinese sources, almost all Sumai 3-based. It is possible that over time the Sumai-3 FHB resistance could break down, which would cause cultivars with Suamai-3 based resistance to become susceptible. Therefore, it is important to identify alternative sources of FHB resistance and so provide breeders with the opportunity to develop cultivars with broad-based resistance to FHB. For this reason, a search for other sources of resistance has been undertaken. The MAS procedure described here was then used to determine if the sources identified were novel or were merely additional sources of the Sumai 3 resistance.